Evaluation of *Gynura procumbens* cutting cultivation using miko-seedcookies

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Abstract. This research was conducted to evaluate the growth and production of *Gynura procumbens* with the miko-seedcookies addition usage. This research was use a randomized block design (RBD) with four treatments of miko-seedcookies diameter which included P0 (control), P1 (4 cm), P2 (5 cm), and P3 (6 cm) and five blocks which included K1, K2, K3, K4, K5. The research used 120 *Gynura procumbens* stem cuttings with an average diameter of 1.30 ± 0.13 cm and a planting length of 19.07 cm. The parameters were the percentage of germinating cuttings, plant height, number of leaves, leaf area, fresh weight, and mycorrhizal colonization. The results showed that the miko-seedcookies utilization increased plant height; number of leaves; fresh weight of shoot, mature leaves, edible stems; dry weight of mature leaves, edible stems; and mycorrhizae colonization. The use 6 diameter of miko-seedcookies could enhance the growth, production, and mycorrhizae colonization continuously.

1 Introduction

Longevity spinach or *Gynura procumbens* (*G. procumbens*) is known as a medicinal plant that grows wild in ditches, shrubs, and grasslands in Southeast Asian regions such as Malaysia, Thailand, and Indonesia, especially in Java Island at an altitude of 1-1200 m ASL and optimally at an altitude of 500 m ASL [1]. *G. procumbens* has great potential to be developed as a feed supplement and antibiotic in animal husbandry because of the active compound content in its leaves. *G. procumbens* leaf extract can inhibit the growth of *E. coli* bacteria [2].

Cultivation of *G. procumbens* with stem cuttings directly in the field is one of the vegetative plant cultivation techniques that is easy to do and less cost [3]. However, the use of this technique often fails because it is influenced by external factors such as soil fertility and weather as well as internal factors such as age and plant genetics [4]. The soil of the previous research site was a latosol soil with a sandy clay loam texture with an acidic soil pH value of 4.62, moderate soil C-organic 3.18%, low N-total 0.27%, moderate P2O5 32.09

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ppm, and low K 0.11 cmol/kg. The soil was dominated by sand 43.47%, dust 23.35%, and clay 33.18% [5]. This showed that the soil was less fertile due to nutrient deficiency.

Miko-seedcookies is an innovative planting media containing arbuscular mycorrhizal fungi (FMA). Arbuscular mycorrhizal fungus is a biological fertilizer as an inoculant made from living organisms that function to tether certain nutrients or facilitate the availability of nutrients in the soil for plants [6]. FMA can increase shoot dry weight production (34.04%), crude protein content (10.21%), phosphorus uptake (40%), N content (10.53%), N uptake (38.10%), and protein production (40.15%) in *Pennisetum purpureum* plants [7]. This planting medium is a place to grow plants in marginal areas such as soils with low productivity, potential and topography that are less favourable for plant growth. Therefore, the potential of *G. procumbens* is important. This study aimed to evaluate the growth and production of *G. procumbens* cultivated with the addition of miko-seedcookies as planting media.

### 2 METHODOLOGY

#### 2.1 Research Procedure

The land used in this study was 12 m x 7.5 m with a plot size of 1 m x 0.5 m and a spacing of 0.5 m x 0.5 m. Cuttings were taken by cutting them with scissors with a length of 25 cm with an average diameter of 1.3 cm and an average mass of 37.36 g. The bottom of the cuttings was cut at an angle of 45°. The bottom of the cuttings was cut at an angle of 45°.

The miko-seedcookies growing medium was made by putting four liters of water into a bucket and adding 10 g of miko-seedcookies adhesive in the form of stiifler and then formed flat rounds like cookies according to the diameter size. *G. procumbens* cuttings were stuck into the miko-seedcookies and penetrated the soil up to one-third of the cuttings.

Maintenance included watering, weeding, and fertilizing. Fertilizers were given in the form of NPK Mutiara 16-16-16 at a dose of 200 kg/ha in week 2 and urea at a dose of 100 kg/ha after four weeks of planting. Harvesting was done at the age of 12 weeks after planting (WAP). *G. procumbens* plants were trimmed to 30 cm and the roots, shoots, mature leaves, and edible stems were taken. The variables observed and measured in this study were the percentage of sprouted cuttings, number of leaves, leaf area, plant height, fresh weight of shoots, mature leaves, and edible stems, also percentage of root colonization.

#### 2.2 Research Design and Data Analysis

This research was carried out in the field with the experimental design used was a randomized blocked design (RBD) with 5 groups of research land positions based on sunlight reception and 4 treatments of miko-seedcookies planting media diameter enriched with arbuscular mycorrhiza, P0 (*G. procumbens* cuttings planted directly without using miko-seedcookies), P1 (using miko-seedcookies with a diameter of 4 cm (53 g)), P2 (using miko-seedcookies with a diameter of 5 cm (84 g)), and P3 (using miko-seedcookies with a diameter of 6 cm (130 g)). Data obtained from the research were analyzed and processed using analysis of variance (ANOVA) with SPSS Statistic 23 software and Microsoft Excel. Analysis of variance that significantly influenced, then further test with Duncan test at 5% level.
3 RESULTS AND DISCUSSIONS

3.1 Growability of *G. procumbens* stem cutting

The percentage of sprouted *G. procumbens* cuttings was not significantly different (p>0.05) after ANOVA test. Data on the percentage of sprouted cuttings was presented in Table 1.

<table>
<thead>
<tr>
<th>Age (WAP)</th>
<th>P0</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>86.67±6.24</td>
<td>96.67±3.33</td>
<td>90.00±6.67</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td>2</td>
<td>93.33±4.08</td>
<td>100.00±0.00</td>
<td>100.00±0.00</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td>3</td>
<td>93.33±4.08</td>
<td>100.00±0.00</td>
<td>100.00±0.00</td>
<td>100.00±0.00</td>
</tr>
</tbody>
</table>

| P0: control (without miko-seedcookies), P1: use of miko-seedcookies diameter 4 cm, P2: use of miko-seedcookies diameter 5 cm, P3: use of miko-seedcookies diameter 6 cm. |

The use of miko-seedcookies did not significantly affect the sprouting percentage of *G. procumbens* cuttings. Cuttings planted without using miko-seedcookies had a high sprouting rate of 93.33% in the third week, while cuttings using miko-seedcookies gave a sprouting percentage of 100% for all sizes. The presence of millet (*Setaria italica*) became a temporary host plant for arbuscular mycorrhizal fungi (FMA) on the miko-seedcookies. The millet would grow on the miko-seedcookies and its roots would first symbiotize with FMA before the roots of *G. procumbens* cuttings were fully formed at the beginning of planting.

3.2 Growth of *G. procumbens* Plant

*G. procumbens* plant growth could be observed through plant height and the number of leaves. The growth of *G. procumbens* could be seen in the graph below (Figure 1).

![Graph of plant height and number of leaves](a) (b)

**Figure 1.** Graph of plant height (a) and number of leaves (b)
The presence of FMA in mico-seedcookes could also increase the absorption of water and macro and micro nutrients. The greater the use of mico-seedcookes, the greater the FMA content which had a positive effect on plant height when compared to without the use of mico-seedcookes. FMA provides nutrient absorption efficiency due to the presence of external hyphae that expanded the nutrient absorption area and accelerated the movement of P to the roots [8].

The use of 6 cm diameter mico-seedcookes (P3) at 12 weeks after planting gave the best number of leaves. The greater amount of FMA causes the absorption area of nutrients, especially nitrogen (N) for the development of plant vegetative organs to be higher. In addition to helping the absorption of macro nutrients, FMA also helped the absorption of micro nutrients such as Zn and Cu which play a role in increasing the growth and development of new shoots [9]. Plant growth and the number of leaves increased if the process of protein synthesis that required nitrogen elements was not inhibited and FMA inoculation increased the absorption of N nutrients by plant roots [10]. After 12 weeks, the number of leaves decreased due to the high intensity of the sun in that week so that many leaves turned yellow and fell off. This is also due to the age of the plant, the higher the age of the plant, the more leaves that fall.

3.3 Production of *G. procumbens* Plant

*G. procumbens* production was evaluated through leaf area and fresh weight at the age of 12 weeks after planting. Leaf area of *G. procumbens* was not significantly different (p>0.05) after ANOVA test. However, the use of mico-seedcookes gave a very significant effect (p<0.01) on the fresh weight of shoots, mature leaves, and edible stems of *G. procumbens* after ANOVA test (Table 2).

| Table 2. Fresh yield of *G. procumbens* plants at the age of 12 WAP |
|------------------|------------------|------------------|------------------|
| **Treatment**    | P0              | P1              | P2              | P3              |
| Fresh yield      |                 |                 |                 |                 |
| Leaf area (mm²)  | 99.94±16.37     | 137.15±23.69    | 119.08±24.88    | 138.80±7.59     |
| Shoot fresh weight (g plant⁻¹) | 5.49±2.60ᵇ       | 6.60±1.92ᵇ      | 7.27±2.23ᵃ      | 7.57±2.65ᵃ      |
| Mature leaves fresh weight (g plant⁻¹) | 24.33±15.96ᵇ     | 31.07±13.41ᵇ    | 44.10±15.62ᵃ    | 51.27±16.41ᵃ    |
| Edible stem fresh weight (g plant⁻¹) | 3.96±4.89ᶜ       | 9.14±8.47ᵇᶜ     | 14.57±10.89ᵇ    | 22.32±15.59ᵃ    |
| Total fresh weight (g plant⁻¹) | 33.79±21.68ᵈ     | 46.81±19.97ᶜ     | 65.93±26.18ᵇ    | 81.15±29.92ᵃ    |

Different superscript letters in the same row indicate highly significant (p<0.01). P0: control (without mico-seedcookes), P1: use of mico-seedcookes diameter 4 cm, P2: use of mico-seedcookes diameter 5 cm, P3: use of mico-seedcookes diameter 6 cm.

The treatment of mico-seedcookes diameter 6 cm (P3) gave a highly significant effect (p<0.01) and the best results for edible stem fresh weight and total fresh weight compared to other treatments. Photosynthesis required sufficient water and nutrients to produce abundant photosynthates. Adequate sunlight also played a role in photosynthesis. FMA could increase photosynthate because the hyphae on FMA could expand the absorption of water and nutrients. The presence of FMA increased the absorption of phosphate (P) which
increased photosynthate which was positively correlated with fresh weight [11]. FMA in miko-seedcookies was effective for the host plant, *G. procumbens*, because it could increase plant dry weight, uptake of P and N nutrients and water used in the photosynthesis process. In a previous study, FMA inoculation increased shoot dry weight production (34.04%), crude protein content (10.21%), phosphorus uptake (40%), N content (10.53%), N uptake (38.10%), and protein production (40.15%) [7].

### 3.4 Root Colonization by Arbuscular Mycorrhizal Fungi

The observation results of FMA colonization on *G. procumbens* roots using 100x magnification microscope were presented in Table 3. The percentage of FMA colonization on *G. procumbens* roots was highly significant different (p<0.01) after ANOVA.

**Table 3. Percentage of root colonization by arbuscular mycorrhizal fungi (%) at 12 WAP**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Treatments</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P0</td>
<td>P1</td>
<td>P2</td>
<td>P3</td>
</tr>
<tr>
<td>Colonization (%)</td>
<td>6.10±6.14c</td>
<td>44.11±11.91b</td>
<td>69.85±8.47a</td>
<td>77.17±17.46a</td>
</tr>
<tr>
<td>Colonization class [12]</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Very high</td>
</tr>
</tbody>
</table>

Different superscript letters in the same row indicate highly significant (p<0.01). P0: control (without miko-seedcookies), P1: use of miko-seedcookies diameter 4 cm, P2: use of miko-seedcookies diameter 5 cm, P3: use of miko-seedcookies diameter 6 cm.

The results of Duncan's further test showed that the treatment of miko-seedcookies diameter 5 cm (P2) and 6 cm (P3) gave a very significant effect (p<0.01) on the percentage of root colonization compared to the control (P0) and the use of miko-seedcookies diameter 4 cm (P1). The larger the size of miko-seedcookies, the percentage of colonization increased because the soil of the research site was less fertile [5] so that FMA in miko-seedcookies encourages more root infection [13]. The association of FMA with *G. procumbens* could be known by the presence of some typical structures in FMA colonization on the roots of its host plant observed through a microscope such as the structure of spores, vesicles, hyphae, and arbuscules (Figure 2).

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Structure of arbuscular mycorrhizal fungi on *G. procumbens* roots (100x magnification). (a): vesicles, (b): spores, (c): hyphae
4 Conclusion

Growth and production of *G. procumbens* increased with the addition of miko-seedcookies in terms of number of leaves, plant height, fresh weight (shoots, mature leaves, and edible stems), and mycorrhizal colonization. The best miko-seedcookies recommended for *G. procumbens* cultivation is 6 cm in diameter.

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Reference